presence of multinucleated cells may be seen in cases of progressive multifocal leukoencephalopathy or in reactive brain tissue around a lymphoma or other infectious process.

HARRY V. VINTERS, MD
Los Angeles

REFERENCES

Anders KH, Guerra WF, Tomiyasu U, et al: The neuropathology of AIDS—UCLA experience and review. Am J Pathol 1986; 124:537-558

Koyanagi Y, Miles S, Mitsuyasu RT, et al: Dual infection of the central nervous system by AIDS viruses with distinct cellular tropisms. Science 1987; 236:819-822

Navia BA, Cho ES, Petito CK, et al: The AIDS dementia complex: II. Neuropathology. Ann Neurol 1986; 19:525-535

Navia BA, Jordan BD, Price RW: The AIDS dementia complex: I. Clinical features. Ann Neurol 1986; 19:517-524

Wiley CA, Schrier RD, Nelson JA, et al: Cellular localization of human immunodeficiency virus infection within the brains of acquired immune deficiency syndrome patients. Proc Natl Acad Sci USA 1986; 83:7089-7093

Papillomavirus in the Lower Female Genital Tract

HUMAN PAPILLOMAVIRUSES (HPV) are classified as genotypes, using molecular hybridization techniques to determine DNA composition, rather than as serotypes based on structural antigens; each genotype has less than 50% genome homology with the other types. At least 46 types of HPV have been identified, 12 of which are associated with lesions of the female genital tract.

Morphologically, condylomas and cervical and vulvar intraepithelial neoplasia may show koilocytotic atypia; this koilocytosis is associated with the presence of HPV. Virus particles have been found in condylomas by electron microscopy, and immunohistochemistry has localized HPV structural antigens to the nuclei of koilocytes.

A number of hybridization techniques may be used to assess tissues for HPV. Southern blot hybridization, applicable to freshly obtained cells and tissues, is the most sensitive and specific technique and can detect less than one copy of HPV genome per cell. Under high and low stringent assay conditions to vary the closeness of the nucleic acid hybrid match, HPV of known and unspecified types can be detected. Dot blot hybridization is a faster but less specific method. In situ filter hybridization was developed for use with freshly exfoliated cells; it also yields more false-positives than the Southern blot technique. In situ tissue hybridization on paraffin-embedded tissue permits specific HPV-infected cells to be identified and localized. With autographic detection using sulfur 35 or tritium, the sensitivity and specificity are similar to those of Southern blotting; with a biotin label, the sensitivity decreases.

Overall, the Southern blot technique has detected HPV DNA in 80% to 90% of cervical condylomas and intraepithelial neoplasia and in 70% to 95% of invasive cervical carcinomas. Differences exist among HPV genotypes in their distribution in the genital tract and in the severity of their associated lesions. In one study, 54% of cervical biopsy specimens of condyloma and intraepithelial neoplasia grade I were associated with HPV type 6 or 11, 13% with type 16, 18, or 31, and 12% with unspecified types; in 21% no HPV was detectable. In contrast, 68% of biopsy specimens of cervical intraepithelial neoplasia grades II and III were positive for type 16, 18, or 31, 12% for type 6 or 11, and 12% for unspecified types, with no HPV detectable in 8%. Other authors have reported comparable trends.

Thus, HPV types 6 and 11 are generally found in ordinary condylomas, although they have infrequently been identified in squamous carcinomas, predominantly of verrucous pattern. Types 16, 18, 31, 33, 35, and 45 are considered to be of higher oncogenic potential and are found mainly in atypical condylomas, moderate and severe dysplasias, and carcinoma in situ and invasive carcinoma of the cervix and vulva. HPV 16 is the most common HPV type identified in invasive cervical squamous cell carcinoma, while type 18 shows a greater association with adenocarcinoma and adenosquamous carcinoma. Preliminary evidence suggests that a progression from condyloma or cervical intraepithelial neoplasia grade I to cervical intraepithelial neoplasia grade III is associated with type 16 or 18 or a mixed infection including one of these types. More than one HPV type are detected in 10% to 20% of patients with carcinoma in situ. Various HPV genotypes, including types 16 and 18, have been detected in 11% of women with normal smears from cervical screening.

There are differences in the physical state of the viral DNA in benign and malignant lesions. Viral DNA is present as extrachromosomal episomes in most preinvasive lesions. Integrated HPV DNA has been noted in dysplasias, invasive carcinomas, their metastases, and cell lines derived from cervical carcinomas.

These findings show important differences among HPV genotypes and strongly suggest that some types of HPV may play a role in carcinogenesis in the lower female genital tract.

SHIRLEY J. HUANG, MD
Los Angeles

REFERENCES

Reid R, Greenberg M, Jenson AB, et al: Sexually transmitted papillomaviral infections—I. The anatomic distribution and pathologic grade of neoplastic lesions associated with different viral types. Am J Obstet Gynecol 1987; 156:212-222

Schneider A, Sawada E, Gissmann L, et al: Human papillomaviruses in women with a history of abnormal Papanicolaou smears and in their male partners. Obstet Gynecol 1987; 69:554-562

Smotkin D, Berek JS, Fu YS, et al: Human papillomavirus deoxyribonucleic acid in adenocarcinoma and adenosquamous carcinoma of the uterine cervix. Obstet Gynecol 1986; 68:241-244

Using the Polymerase Chain Reaction in Detecting Infectious Agents

THE DIAGNOSIS OF INFECTIOUS DISEASES is an important function of pathologists. Because most microorganisms are difficult or impossible to detect or because they speciate on histologic examination, a definitive diagnosis usually depends on culturing. This, however, can be an expensive and time-consuming process, and methods for culturing certain microorganisms are not available. Furthermore, fresh tissue is needed.

In 1985 a new technique of DNA amplification, known as the polymerase chain reaction, was described. With this method, any DNA fragment of known sequence can be selectively amplified and subsequently determined by either dot or liquid hybridization. The DNA does not have to be purified and can even be directly obtained from deparaffinized, thick sections of formalin-fixed, paraffin-embedded material. Therefore, the presence of any infectious agent in tissue or body fluid can be established with sensitivity and specificity, provided that a portion of its DNA sequence is known. This method has been successfully applied to the identification of the human immunodeficiency virus in blood and the human papillomavirus in cervical carcinoma specimens.

The method as described above, however, still involves hybridization using probes labeled with phosphorus 32; therefore, it is not suitable for routine clinical use. Recently the procedure has been improved to the extent that the amplified DNA product can be directly shown as a visible band on an agarose gel after electrophoresis and staining with ethidium bromide. We have successfully used this protocol to

identify hepatitis B virus in serum specimens and herpesvirus in paraffin blocks of specimens of infected skin, with 100% sensitivity and specificity. With this modified technique, the entire procedure can be done in less than six hours, with no need for radioisotopes. Therefore, the polymerase chain reaction offers a rapid, sensitive, and specific technique for detecting infectious agents that can use both fresh and fixed material and in the future may be increasingly used in clinical laboratories for diagnostic purposes.

T.S. BENEDICT YEN, MD, PhD
San Francisco

REFERENCES

Cao M, Xiao X, Egbert B, et al: Rapid detection of herpes simplex virus infection with the polymerase chain reaction. J Invest Dermatol, in press

Kwok S, Mack DH, Mullis KB, et al: Identification of human immunodeficiency virus sequences by using in vitro enzyme amplification and oligomer cleavage detection. J Virol 1987; 61:1690-1694

Shibata DK, Arnheim N, Martin WJ: Detection of human papillomavirus in paraffin-embedded tissue using the polymerase chain reaction. J Exp Med 1988; 167:225-230

Diagnosis of Small Round Cell Tumors in Children

THE TERM "SMALL, ROUND, BLUE CELL TUMORS" refers collectively to a group of several aggressive malignant disorders of childhood of diverse histogenesis that are capable of presenting histologically as proliferations of small primitive cells with round nuclei and undifferentiated cytoplasm. The most important of these are lymphoma, Ewing's sarcoma, rhabdomyosarcoma, and neuroblastoma, although a number of other disorders are occasionally considered in the differential. While most cases of each of these neoplasms can be diagnosed by conventional histopathology, some of these tumors elude identification by routine light microscopy alone. Fortunately, several ancillary techniques, including sophisticated applications of recent advances in immunology, genetics, and molecular biology, are available for resolving problematic cases.

Electron microscopy will often provide an answer in these cases by revealing neuritic processes in neuroblastoma, actin-myosin bundles in rhabdomyosarcoma, glycogen pools and a general lack of cytoplasmic differentiation in Ewing's sarcoma, an absence of true cell attachments in lymphoma, and various other features. Immunocytochemistry has proved its value in the diagnosis of small, round, blue cell tumors. The detection of myoglobin or desmin or both in primitive tumor cells may confirm the diagnosis of rhabdomyosarcoma, while a positive reaction for neuron-specific enolase may point towards neuroblastoma or other primitive neural tumors. Newer monoclonal antibodies against muscle-specific actin and various neural antigens such as Leu-7 and HSAN 1.2 are providing additional help. The demonstration of leukocyte-common antigen may be invaluable in the diagnosis of lymphoma in an unusual site, while a battery of more specific markers may aid in its precise classification.

The most exciting recent advances in the diagnosis of small, round, blue cell tumors have been in the study of tumor cell chromosomes and DNA by cytogenetic and molecular genetic methods. Specific chromosomal abnormalities have been found in certain tumors, such as a reciprocal 11:22 translocation in Ewing's sarcoma. The finding of the same translocation in peripheral neuroepithelioma raises fascinating histogenetic possibilities. Studies of oncogene amplification and expression in small, round, blue cell tumors, such as the N-*myc* oncogene in neuroblastoma, have been shown to provide useful diagnostic and prognostic information and to shed light on tumorigenesis. These and other newer developments bring us closer to our goal of a precise diagnosis in every case of small, round, blue cell tumor.

DOUGLAS A. WEEKS, MD
Loma Linda, California

REFERENCES

Brodeur GM, Seeger RC, Schwab M, et al: Amplification of N-*myc* in untreated human neuroblastomas correlates with advanced disease stage. Science 1984; 224:1121-1124

Mierau GW, Berry PJ, Orsini EN: Small round cell neoplasms: Can electron microscopy and immunohistochemical studies accurately classify them? Ultrastruct Pathol 1985; 9:99-111

Triche TJ, Askin FB, Kissane JM: Neuroblastoma, Ewing's sarcoma, and the differential diagnosis of small-, round-, blue-cell tumors, *In* Finegold M (Ed): Pathology of Neoplasia in Children and Adolescents. Philadelphia, WB Saunders, 1986, pp 145-195